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Effects of Ingestion of Ponderosa Pine Needles by Late-Pregnant Cows on Uterine Blood Flow and Steroid Secretion^{1,2}

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ABSTRACT: Consumption of Ponderosa pine needles by late-pregnant beef cows results in the premature delivery of a viable calf. We have demonstrated the presence of a factor(s) in plasma from cows fed pine needles that specifically increased uterine arterial tone (i.e., decreased arterial diameter) in vitro. This study was designed to investigate changes in uterine blood flow and steroid secretion/uptake by the gravid uterus of cows fed pine needles to induce premature parturition. Sixteen beef cows were laparotomized on d 240 of gestation, and an electromagnetic blood-flow probe was placed around the uterine artery supplying the gravid horn. Cows were randomly assigned on d 250 of gestation to a control (n = 8; 8.2 kg/d of alfalfa hay) or pine needle (n = 8; 2.7 kg/d of pine needles + 5.5 kg/d of alfalfa hay) diet. Uterine blood flow was monitored, and systemic blood (uterine arterial and/or jugular venous) and uterine venous blood samples were collected daily between 0630 and 0800, just before feeding. Five of eight cows fed pine needles calved prematurely (average day of gestation = 260.2 ±

.6) compared with cows fed the control diet, which calved on 287.6 ± 3.4 d of gestation. Uterine blood flow in the control cows remained constant from d 250 through the day of parturition. In contrast, uterine blood flow of cows fed pine needles that calved early decreased progressively ($P < .01$), declining to 25.2% of its original value by the day of parturition.

Cows fed the pine needle-diet and cows fed the control diet exhibited similar declines in progesterone concentrations in systemic blood and uterine venous blood during the last 4 d of pregnancy. Although estrone and estradiol-17 β both increased during the last 9 d preceding parturition in both cows fed the control diet and those fed the pine needle diet, cows fed pine needles exhibited reduced ($P < .002$) concentrations of both estrogens in both systemic and uterine venous blood. We conclude that consumption of pine needles progressively reduces uterine blood flow to the gravid horn and that this reduction induces the onset of a premature parturition as evidenced by normal prepartum changes in steroid secretion.

Key Words: Pine Needles, Parturition, Uterus, Blood Flow, Cows

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Introduction

Reports by James et al. (1977), Short et al. (1987), and Stevenson et al. (1972) suggested that ingestion of *Pinus ponderosa* pine needles induced premature parturition as evidenced by the birth of a weak but viable calf. Jensen et al. (1989) and Stuart et al. (1989) failed to confirm a bacterial, fungal, chlamydial, or viral cause for pine needle-induced premature parturition and were unable to find any specific lesions in the dam or newborn calf. However, Stuart et al. (1989) described vessels

within the caruncular arterial bed of pine needle-fed cows at necropsy as notably reduced in diameter in association with an ischemic necrosis of the central portion of the caruncles. Further, Christenson et al. (1992) observed that blood plasma from cows fed pine needles contained a component(s) that caused a long-term reduction in uterine arterial diameter when infused into the caruncular artery of an isolated bovine placentalome.

Blood flow through the uterine arterial bed is affected by two Ca^{++} -dependent contractile mechanisms, phasic contractility and tone, which seem to be modulated separately by steroid hormones (Ford, 1989). Phasic contractility remains intact throughout gestation, allowing transient reductions (3 to 10 min) in uterine blood flow (UBF) during periods of acute maternal stress that are not detrimental to fetal well-being (Shnider et al., 1979; Sauer et al., 1989). Uterine arterial smooth muscle tone sets the baseline rate of blood flow to the uterus throughout gestation (Ford, 1989). Progressive declines in uterine arterial tone allow UBF to increase, ensuring an adequate supply of nutrients to the rapidly growing fetus (Reynolds et al., 1986).

Based on these data, it was hypothesized that pine needle consumption might decrease UBF, thereby prompting the initiation of a premature parturition. The objective of this experiment therefore was to compare changes in UBF and steroid secretion/uptake by the gravid uterus in a group of cows fed pine needles that calved prematurely and a group of cows fed a control diet.

Materials and Methods

Eleven multiparous beef cows and two beef heifers of mixed breeding with known breeding dates were transported from Fort Keogh Livestock and Range Research Laboratory, Miles City, MT, to the Animal Reproduction farm at Ames, IA, 2 mo before their assignment to the experiment. Because of previous experience that suggested that several control cows would be lost from the study due to the prolonged interval from surgery to parturition (e.g., failure of catheters and/or flow probes), several additional cows from the Ames herd were maintained as replacements. Three of these cows from the Ames herd were used to replace three control cows lost from the experiment.

Cows and heifers were adjusted to handling and confinement for at least 3 wk before surgery. During this period, cows were continually maintained in a paddock area, except during daily feeding periods when cows were placed in individ-

ual stanchions and fed approximately 8.2 kg/d of alfalfa hay. Feed was withheld for 48 h and water for 24 h before surgery, which was performed on each animal between d 239 and 244 of pregnancy. Anesthesia was induced and maintained as previously described (Reynolds et al., 1983). The cow was then positioned in dorsal recumbency, and the gravid uterus was exposed via a midventral laparotomy. The reproductive tract was then manipulated to gain access to the main uterine artery supplying the gravid horn. After visualization of the artery, an electromagnetic blood-flow probe (Model 501D; Carolina Medical Electronic, King, NC) of the correct luminal circumference (20, 25, or 30 mm) was selected. The blood-flow probe and two heparinized polyvinyl catheters (1.12-mm i.d., 1.65-mm o.d.) to be used for collection of uterine arterial and venous blood were inserted into the body cavity through a small puncture wound in the flank. Because the main uterine artery in four cows had a circumference > 30 mm, which exceeded the size of the blood-flow probes, a large branch of the uterine artery perfusing the gravid uterus was used in these animals. After placement of the probe around the main uterine artery or a large branch, it was secured in position by silk ligatures to prevent slippage or displacement. Catheters were then inserted into a small distal branch of the uterine artery and uterine vein. Between blood samplings, catheters were filled with heparinized saline (100 IU of heparin/mL) that contained 1% penicillin-dihydrostreptomycin (Combiotic, Pfizer, New York) by volume. The exteriorized portions of the catheters and blood-flow probe were then secured to the flank with elastic tape and tag cement. Recovery of cows after surgery was rapid; the animals generally stood, ate feed, and drank water within 1 h. Immediately after surgery, and for the next 3 d, the cows were given penicillin-dihydrostreptomycin (Pfizer) according to body weight.

Feeding Regimen. Twelve hours after surgery, cows were returned to their outside pen and thereafter were individually stanchioned and fed twice daily from 0800 to 1200 and from 1530 to 1830. Animals continued to receive a total of 8.2 kg/d of alfalfa hay in the same stanchion they had been assigned during the adjustment period. Water and a vitamin-mineral supplement (Master Mix All Purpose Min-Plus, Central Soya, Fort Wayne, IN) were available at all times except when cows were stanchioned for feeding and monitoring. *Pinus ponderosa* pine needles used in this study were from the same batch used by Short et al. (1987) and were known to induce premature parturition in late-pregnant beef cows. To enhance the consumption of pine needles, all cows, regardless of diet, were restricted to half their previous daily ration

on d 248 of pregnancy and received no feed on the day before the initiation of the control or pine needle diets. On d 250 of pregnancy, cows were then assigned to receive either the control diet ($n = 8$; 8.2 kg of chopped alfalfa hay) or the pine needle diet ($n = 8$; 5.5 kg of chopped alfalfa hay + 2.7 kg of chopped pine needles). To facilitate uniform consumption of alfalfa and pine needles, the alfalfa hay and pine needles were chopped separately into 2- to 5-cm particles and then mixed just before feeding. The pine needle-fed cows were given half their hay and all the pine needles in the morning, whereas during the afternoon the remaining one-half of the hay was mixed with any material left from the morning feeding. Cows fed the control diet were handled similarly, receiving two-thirds of their chopped alfalfa hay in the morning and the remaining hay in the afternoon. Orts were weighed each evening for all cows. Three of eight cows failing to calve within 14 d after the initiation of pine needle feeding were removed from the study. This interval of pine needle feeding was selected because the research by Short et al. (1987, 1989) demonstrated that virtually all pine needle-induced premature parturitions had occurred by that time. Because this study was initiated to evaluate changes preceding pine needle-induced parturition, only data from the five cows calving within 14 d of the initiation of pine needle feeding are compared with data obtained from eight cows fed the control diet.

Monitoring of Uterine Blood Flow and Collection of Blood Samples. Throughout the surgical recovery period (d 240 to 249 of pregnancy), catheters were checked daily for patency and blood-flow probes for functionality. In 4 of 16 cows, uterine arterial catheter patency was lost before assignment to the study, and it was therefore decided to use jugular venous blood to estimate systemic steroid levels for these animals. No differences were observed when uterine arterial and jugular venous concentrations of progesterone ($7.0 \pm .9$ and 8.6 ± 1.1 ng/mL), estrone (873 ± 95 and 860 ± 105 pg/mL), or estradiol-17 β (134 ± 25 and 162 ± 36 pg/mL) were compared within two control cows from which daily samples were collected from both vessels throughout the sampling period. Beginning on d 250 and continuing throughout the study, the UBF of each cow was monitored continuously for a 10-min period immediately before the morning feeding (0630 to 0800). Values were recorded every 15 s throughout the 10-min period, and the average of these measurements was considered an estimate of UBF for that day. Six of eight cows fed the control diet and four of five cows fed the pine needle diet that calved prematurely had functional blood flow transducers throughout the experimental period. Only UBF data from these cows are

presented.

Immediately before or after UBF recordings, 10 mL of uterine arterial and uterine venous blood was collected by allowing catheters to drip into 16-mm \times 100-mm tubes containing .2 mL of heparinized saline (100 IU of heparin/mL) as an anticoagulant. Jugular venous samples were also collected from all animals by venipuncture and similarly placed into heparinized tubes. After collection, blood samples were immediately centrifuged, and plasma was collected and frozen at -20°C until it was analyzed for progesterone, estrone, and estradiol-17 β by RIA.

Progesterone was assayed as described and validated by Reynolds et al. (1983). Interassay variation was determined by inclusion of a plasma pool from a late-pregnant cow in each assay ($n = 10$). Intraassay variation was determined by inclusion of replicates ($n = 4$) of the plasma pool within one assay. The interassay and intraassay CV for progesterone were 9.9 and 3.1%, respectively. Assays for estrone and estradiol-17 β were as described and validated by Ferrell et al. (1983). Interassay ($n = 8$) and intraassay variation ($n = 6$) were determined on a plasma pool from a late-pregnant cow as described for progesterone. Interassay and intraassay CV were 8.9 and 6.3% for estrone and 6.7 and 3.9% for estradiol-17 β .

Statistical Analysis. Daily UBF values within a cow were converted to a percentage change from the d 250 UBF value for that cow to make comparisons across cows. This was considered necessary because absolute UBF values on d 250 reflected differing amounts of blood flow to the gravid horn (i.e., whether the flow probes were placed on the main uterine artery or a major branch). Percentage changes in uterine blood flow and steroid concentrations were analyzed as a completely randomized split-plot design with cows forming the main plots and day forming the subplots. Treatment, day, and treatment \times day effects were tested using the appropriate error terms. After observation of a significant ($P < .05$) *F*-test, comparisons within treatment groups were made using a conservative *t*-statistic (Cochran and Cox, 1957). Correlation coefficients between amount (kilograms) of diet consumed and incidence and time to premature parturition were calculated for the pine needle-fed cows.

Results

After initiation of pine needle feeding, five of the eight cows (65.2%) gave birth to live calves 10.2 \pm .6 d later (\approx 260 d of pregnancy). The amount of pine needles consumed was not correlated with the incidence of a premature parturition or with

the interval from the initiation of pine needle feeding to parturition among the five cows that calved prematurely. All pine needle-fed cows that calved prematurely exhibited a normal sequence of visible preparturient changes (i.e., development of mammary gland, relaxation of the pelvic ligaments, swelling of the genitalia, and increased mucus secretion from the vulva) beginning approximately 3 to 4 d before parturition. Additionally, all pine needle-fed cows that calved retained the fetal membranes for > 48 h. All control-fed cows had normal gestation lengths (287.6 ± 3.4 d) and none exhibited retention of fetal membranes for > 12 h.

No changes in UBF were detected from d 250 through the day of parturition for cows fed the control diet (Figure 1). In contrast, pine needle-fed cows that calved prematurely exhibited a decrease ($P < .05$) in UBF by d 4 of pine needle feeding. Uterine blood flow continued to decline in a linear fashion during the next 4 d, to reach $54.9 \pm 10.7\%$ of the initial value. On the day of parturition, UBF was 74.8% less than that observed on d 250 in the two pine needle-fed cows for which day of parturition recordings were obtained. The other two cows calved before the monitoring period on the day of parturition, and thus no UBF values were obtained.

Analysis of systemic and uterine venous progesterone concentrations and patterns of change (i.e.,

treatment \times day interaction) showed no differences between treatment groups (Figures 2 and 3). Throughout the sampling period, systemic progesterone concentrations were as high or higher than those observed in uterine venous blood in both cows receiving the control diet and those receiving the pine needle diet. Further, progesterone concentrations were maintained at a relatively constant level in both treatment groups until 4 d before parturition, at which time control-fed and pine needle-fed cows exhibited a progressive decline in progesterone concentrations, reaching the lowest levels on the day of parturition.

Because of parallel profiles of estrone and estradiol-17 β throughout the last 9 d of gestation for each cow, concentrations of estrone and estradiol-17 β were summed for each sample and referred to as total estrogen (Figure 4). The patterns of change of estrogens for both systemic and uterine venous blood were similar across both treatment groups. Uterine venous estrogen concentrations were greater than those observed in systemic blood for both pine needle-fed and control-fed cows. However, estrogen concentrations were lower ($P < .002$) in pine needle-fed than in control-fed cows. Ratios of systemic estrogen:progesterone over the 9 d before parturition showed a similar pattern of change for both treatment groups, but the absolute values were 2 to 6 times greater ($P < .02$) in the control-fed cows than in pine needle-fed cows.

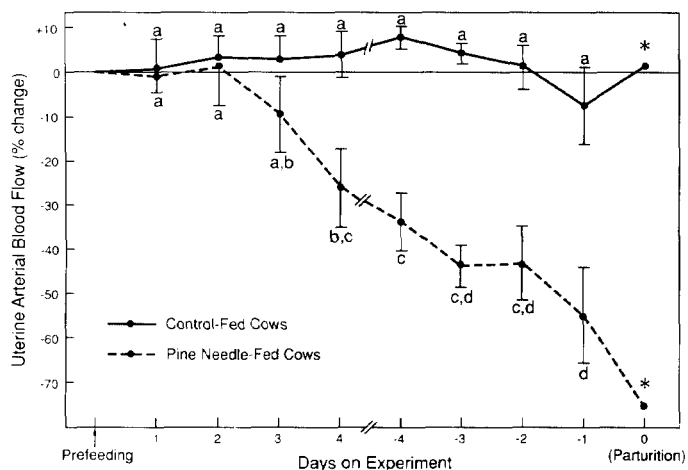


Figure 1. Uterine blood flow (percentage of change from the prefeeding value) for the control-fed cows and Ponderosa pine needle-fed cows that calved early during the 4 d after initiation of the diets and the 4 d preceding parturition. ^{a,b,c,d}Means within treatments with different superscripts differ ($P < .05$). Each mean \pm SE represents values from six control-fed cows or four pine needle-fed cows. *Mean of two cows that calved after uterine blood flow recordings on the day of parturition.

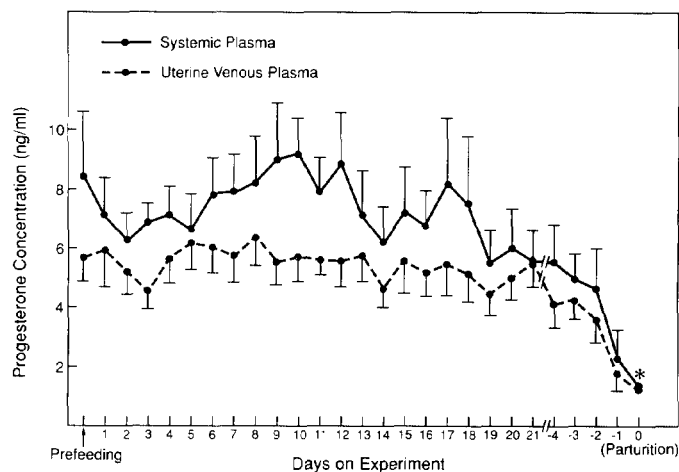


Figure 2. Systemic and uterine venous plasma progesterone concentrations from d 250 of gestation through parturition for control-fed cows. Means \pm SE represent systemic plasma from five cows and uterine venous plasma from seven cows. *Mean of two cows that calved after blood collection on the day of parturition.

Discussion

Consumption of Ponderosa pine needles caused a marked, progressive reduction in UBF followed by premature parturition in five of eight cows. In contrast, UBF of control-fed cows remained relatively constant from d 250 through the day of parturition, which is consistent with previous results during late gestation in the ewe or cow (Caton et al., 1980; Ford et al., 1982; Guilbault et al., 1984). The progressive decline in UBF observed in cows after the initiation of feeding pine needles supports *in vitro* data (Christenson et al., 1992) demonstrating the presence of a vasoactive substance in plasma of cows fed pine needles that increased caruncular arterial tone. In the ewe and cow, nutrient and O_2 delivery to the fetus is directly dependent on the rate of UBF (Alexander, 1964; Ferrell and Ford, 1980; Reynolds et al., 1986). Evidence that reductions in UBF may facilitate an early parturition was reported in late-pregnant ewes, in which acute reductions in UBF stimulated fetal cortisol release, which is thought to initiate parturition in both ewes and cows (Bazer and First, 1983; Challis et al., 1989).

As previously stated, uterine arterial smooth muscle tone controls long-term changes in the rate of uterine blood flow. Specific metabolites of estrogen (catechol estrogens) seem to regulate uterine arterial tone by inhibiting extracellular uptake of Ca^{++} through potential-sensitive Ca^{++} channels (Stice et al., 1987a). This Ca^{++} channel blockade results in the reduced ability of the

uterine arterial smooth muscle cell to sustain a contraction in response to circulating concentrations of catecholamines and is indicative of the changes observed to occur during pregnancy (Sauer et al., 1989). It is possible that a component in pine needles or a metabolite of it in blood (Christenson et al., 1992) inhibits enzymatic conversion of endogenous estrogens to catechol estrogen, or, alternatively, serves as a potential-sensitive Ca^{++} channel agonist that directly opens the potential-sensitive Ca^{++} channel, allowing sustained reductions in arterial diameter to occur.

Studies conducted in rodents support the presence of a compound(s) in pine needles that has antiestrogen and/or anticatchol estrogen-like activity. Allen and Kitts (1961), Allison and Kitts (1964), and Cook and Kitts (1964) demonstrated

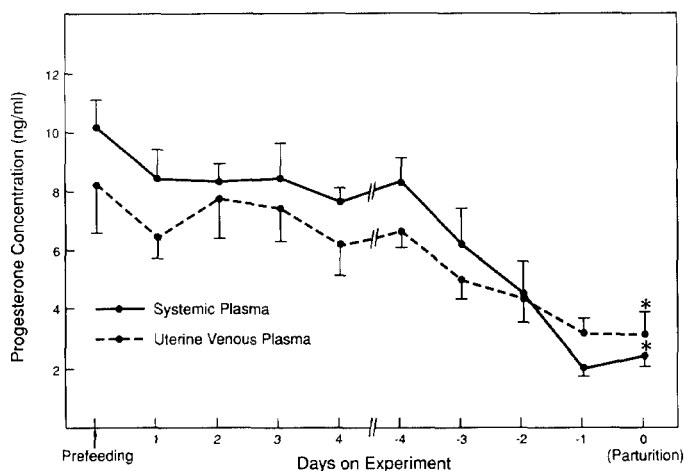


Figure 3. Systemic and uterine venous plasma progesterone concentrations from d 250 of gestation through parturition for the cows fed ponderosa pine needles. Means \pm SE represent systemic and uterine venous plasma obtained from five pine needle-fed cows. *Mean \pm SE of three cows that calved after blood collection on the day of parturition.

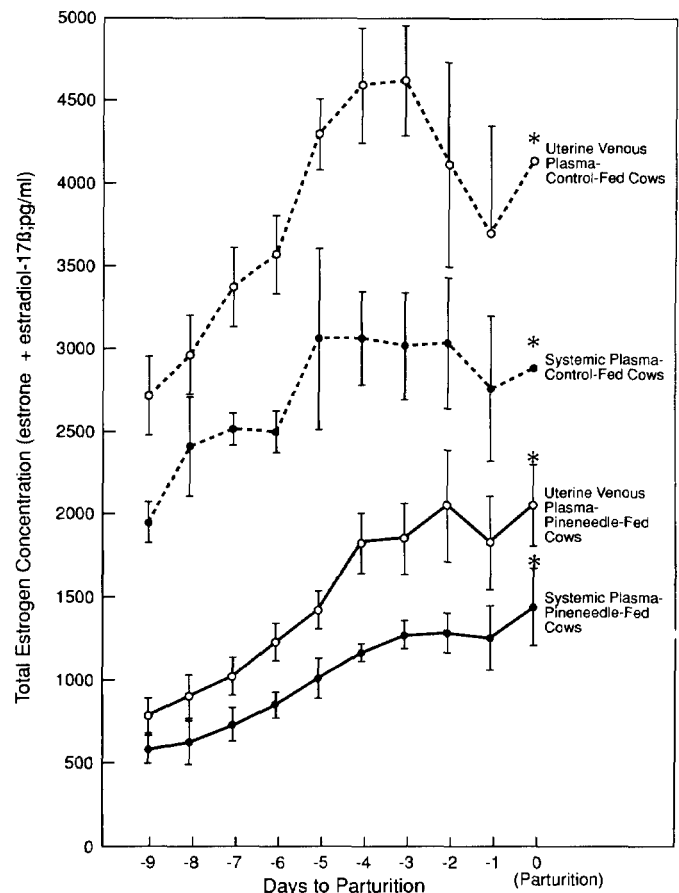


Figure 4. Estrogen concentrations in systemic and uterine venous plasma in both control-fed cows and those fed ponderosa pine needles during the last 9 d of gestation. Means \pm SE represents systemic and uterine venous plasma obtained from five and seven control-fed cows, respectively, or five pine needle-fed cows. *Mean of two control-fed cows or mean \pm SE of three pine needle-fed cows that calved after blood collection on the day of parturition.

that an organic extract of pine needles injected simultaneously with estradiol-17 β could prevent the uterine hyperemic response initiated by estrogen administration. Later, Wagner and Jackson (1983) found that a component of pine needles could effectively inhibit estradiol-17 β binding in a receptor binding study. In addition, studies have shown that catechol estrogens compete effectively with estradiol-17 β for specific receptor binding sites in the rat uterine cytosolic binding assay (Martucci and Fishman, 1979).

In agreement with the results of Short et al. (1989), estrogen concentrations in systemic blood increased progressively after the initiation of pine needle feeding. Further, in both the current study and that of Short et al. (1989), the changes in estrogen preceding parturition were similar to those of cows fed a control diet. Therefore, the normal rise in estrogen associated with parturition was initiated early by feeding pine needles. This gradual rise in estrogen concentration, with maximal values at parturition, has been reported previously for normal parturition (Smith et al., 1973) as well as for a parturition induced by prostaglandins (Henricks et al., 1977) or corticoids (Chew et al., 1978). In contrast to the results of Short et al. (1989), however, estrogen concentrations in systemic blood of pine needle-fed cows in this study were reduced compared with those in systemic blood of control-fed cows during the prepartum period. This seems to result from a decreased secretion of estrogen by the gravid uterus, as evidenced by the markedly reduced uterine venous concentrations in pine needle-fed cows (Figure 4).

Results of this study also contrast with those of Short et al. (1989) in that they fail to demonstrate that feeding pine needles elevates systemic progesterone concentrations. When progesterone data were summarized by day before parturition for pine needle-fed and control-fed cows, concentrations were similar throughout the prepartum period. The pattern of progesterone in systemic blood during the last few days of pregnancy was similar to that observed preceding a normal parturition (Smith et al., 1973) or a parturition induced by prostaglandins (Henricks et al., 1977) or corticoids (Chew et al., 1978). The acute increase in circulating progesterone concentrations observed by Short et al. (1989) after the initiation of pine needle feeding may have resulted from adrenal progesterone release due to the stress of handling, feeding, and bleeding untrained range cows. Wiersma and Stott (1969) demonstrated increased blood progesterone after thermal stress in cows with or without ovaries. Further, when tissues were obtained from thermal-stressed and control cows, it was observed that ovarian tissue proges-

terone levels were similar for all cows, whereas adrenal progesterone concentrations were elevated in the thermal-stressed group. Furthermore, Wendorf et al. (1983) demonstrated that bilateral adrenalectomy-ovariectomy of cows on d 215 of gestation resulted in the rapid disappearance (24 h) of progesterone from systemic blood, and all cows aborted 3 to 6 d later. In contrast, bilateral ovariectomized cows maintained low circulating levels of progesterone with no reduction in gestation length.

The high incidence of retained placentas in treated cows in this study may result from the exposure of the gravid uterus to a decreased estrogen:progesterone ratio in systemic blood. This hypothesis is consistent with previous research demonstrating that placental retention was associated with a reduced estrogen:progesterone ratio in blood, whether the cows were induced to calve prematurely with dexamethasone (Chew et al., 1978, 1979) or calved after a normal gestation length (Chew et al., 1977). This altered estrogen:progesterone ratio may result in a reduced uterine motility and/or may inhibit detachment of the placenta from the uterine wall (Garfield et al., 1979).

Implications

These data support the presence of a factor(s) in the blood of cows consuming pine needles during late pregnancy that progressively decreases uterine blood flow, resulting in the premature birth of a weak, viable calf. This induced premature parturition seemed normal in that it was associated with normal prepartum changes in steroid profiles, mammary development, relaxation of the pelvic ligaments, swelling of the genitalia, and increased mucus secretion from the vulva.

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